

RESEARCH PAPER

A comparison of the pharmacological profiles of prasugrel and ticagrelor assessed by platelet aggregation, thrombus formation and haemostasis in rats

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BACKGROUND AND PURPOSE

Prasugrel is a third-generation thienopyridine prodrug and ticagrelor is a non-competitive P2Y₁₂ receptor antagonist. In their phase 3 studies, both agents reduced rates of ischemic events relative to treatment with clopidogrel.

EXPERIMENTAL APPROACH

The pharmacodynamic profile of anti-platelet effects of prasugrel was compared with that of ticagrelor in rats.

KEY RESULTS

The active metabolite of prasugrel was less potent than ticagrelor and its active metabolite on platelet aggregation *in vitro*. In contrast, prasugrel was a more potent antiplatelet agent than ticagrelor on *ex vivo* platelet aggregation: their ED₅₀ values at peak for ADP 20 µmol·L⁻¹ were 1.9 and 8.0 mg·kg⁻¹, respectively. Prasugrel's inhibition of platelet aggregation was maintained for up to 24 h after administration, but ticagrelor's duration of action was substantially shorter. Prasugrel and ticagrelor significantly inhibited thrombus formation with ED₅₀ values of 1.8 and 7.7 mg·kg⁻¹, respectively. Both agents also prolonged bleeding times (ED₂₀₀ values of 3.0 and 13 mg·kg⁻¹ respectively) suggesting that at equivalent levels of inhibition of platelet aggregation, the agents would show comparable antithrombotic activity with similar bleeding risk. Platelet transfusion significantly increased blood platelet numbers similarly in prasugrel- and ticagrelor-treated rats. In the prasugrel-treated group, platelet transfusion caused significant shortening of bleeding time, while in the ticagrelor-treated group, platelet transfusion showed no influence on bleeding time under the experimental conditions employed.

CONCLUSIONS AND IMPLICATIONS

Prasugrel and ticagrelor showed several differences in their pharmacological profiles and these disparities may reflect their differing reversibility and/or pharmacokinetic profiles.

Abbreviations

ACS, acute coronary syndrome; PCI, percutaneous coronary intervention; PRP, platelet-rich plasma

Introduction

Prasugrel is a third generation thienopyridine antiplatelet prodrug (Niitsu *et al.*, 2005; Jakubowski *et al.*, 2007), requiring *in vivo* metabolism to generate the active metabolite R-138727 that is a specific and irreversible antagonist of the platelet P2Y₁₂ ADP receptor (Sugidachi *et al.*, 2001; 2007; receptor nomenclature follows Alexander *et al.*, 2011). Prasugrel has the potential to provide both more consistent and greater blockade of P2Y₁₂ receptors than clopidogrel (Dobesh, 2009). The better pharmacokinetics and pharmacodynamics of prasugrel compared with clopidogrel result not only in more effective platelet inhibition, but greater clinical benefits in acute coronary syndrome (ACS) patients undergoing percutaneous coronary intervention (PCI; Wiviott *et al.*, 2007; Li *et al.*, 2009).

Ticagrelor, a cyclopentyl-triazolo-pyrimidine, is a new chemical class of non-competitive and reversible P2Y₁₂ receptor antagonist (van Giezen *et al.*, 2009). Ticagrelor was recently approved for use in ACS patients (Wijeyeratne *et al.*, 2012) based on its phase 3 study, (PLATO, platelet inhibition and patient outcomes) which showed a significant benefit compared with clopidogrel (Wallentin *et al.*, 2009). As with the thienopyridines, ticagrelor undergoes CYP-mediated metabolism to produce an active metabolite, AR-C124910XX (Teng and Butler, 2010), but in contrast to the thienopyridines, both the parent drug and active metabolite exhibit platelet inhibitory activity (van Giezen and Humphries, 2005; Teng and Butler, 2010). Pharmacological assessment of treatment with ticagrelor must therefore consider the pharmacokinetics and pharmacodynamics of both ticagrelor and its active metabolite.

Both prasugrel and ticagrelor were more effective than clopidogrel and may therefore be preferred in ACS patients. However, to date, no large-scale clinical study has directly compared the efficacy and safety of prasugrel and ticagrelor (Alber *et al.*, 2011), although a small clinical pharmacology study in ACS patients with high on-treatment platelet reactivity (Alexopoulos *et al.*, 2012) and an adjusted indirect meta-analysis comparing both agents (Biondi-Zoccai *et al.*, 2011) have been reported. Moreover, there is, so far, no detailed non-clinical comparison of the pharmacological profile of prasugrel and ticagrelor.

In the present study, we determined the relative pharmacological profiles of the anti-platelet activities of prasugrel and ticagrelor, using experimental models in rats, and found several differences. To our knowledge, this is the first formal, detailed non-clinical study to compare the pharmacological profiles of these novel P2Y₁₂ receptor antagonists.

Methods

Experimental animals

All animal care and experimental procedures complied with the institutional 'Animal experiment ethical code' (NISSEI BILIS Co., Ltd. and the Safety Research Institute for Chemical Compounds Co., Ltd) and the guidelines of the Institutional Animal Care and Use Committee (Daiichi Sankyo Co., Ltd). All studies involving animals are reported in accordance with the ARRIVE guidelines (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010). A total of 457 animals were used in the experiments described here. Male Sprague-Dawley rats (Japan SLC, Inc.,

Shizuoka, Japan; 7 weeks old at receipt) were used in the present study. Rats were housed in animal quarters set at a constant temperature, humidity and 12 h light/dark cycle. Rats were maintained with free access to water and food and used after an acclimatization period of at least 6 days.

Preparation of platelet-rich plasma (PRP) and platelet-poor plasma (PPP)

In *ex vivo* studies, blood was collected from the abdominal aorta under anaesthesia with pentobarbital sodium (35 mg·kg⁻¹, i.p.) at 1, 2, 4, 8, 12 and 24 h after the administration of the test agent. 4.5 mL of blood was drawn into a disposable syringe containing 0.5 mL of 3.8% sodium citrate (pH 7.4). For *in vitro* studies, 6 mL of blood was collected in a similar manner. The anti-coagulated blood was centrifuged (150–200× *g* for 10 min at room temperature) to obtain PRP. After obtaining PRP, the remaining blood was centrifuged (1300–1500× *g* for 15 min at room temperature) to obtain PPP. Platelet counts in the PRP were obtained using an automated blood cell counter (F-800 or XT-2000 iV, Sysmex Corporation, Hyogo, Japan), and PRP containing $50 \pm 5 \times 10^4$ platelets·μL⁻¹ was prepared by diluting with PPP.

Platelet aggregation

In the *in vitro* studies, PRP was incubated with the vehicle or the test agents for 30 min at room temperature before measuring aggregation to ADP. In the *ex vivo* studies, 240 μL of the PRP prepared was stirred for 1 min at 37°C, and 10 μL of agonist (ADP or collagen) was subsequently added to induce platelet aggregation. Platelet aggregation was monitored for 10 min after agonist addition and recorded as maximum platelet aggregation using a 12-channel automated platelet aggregometer (MCM HEMA TRACER 313 or 712, MC Medical, Inc., Tokyo, Japan).

Arterio-venous (AV) shunt thrombosis model

The ability of the agents to prevent thrombus formation was assessed using an AV shunt model described previously by Sugidachi *et al.* (2000) with slight modifications. After anaesthesia with pentobarbital sodium (50 mg·kg⁻¹, i.p.), the jugular vein and contralateral carotid artery of rats were exposed and were cannulated with a tube that contained a 10 cm long silk thread in its lumen and filled with heparinized saline (30 units·kg⁻¹). Blood circulation was started through the shunt, initiating thrombus formation on the silk thread 4 h after administration of test agents. After blood circulation for 30 min, the shunt was removed from the vein, the rat killed by exsanguination and the thread was carefully removed from the tube. After blotting the thread with a filter paper, the wet weight of the thread was determined, and the thrombus weight was calculated by subtracting the original weight of the silk thread. An electronic analytical balance (AX200; Shimadzu Corporation, Kyoto, Japan) was used for measuring the weight of the thrombus.

Measurement of bleeding time

Four hours after administration of vehicle, prasugrel or ticagrelor, rats were placed in a rodent restrainer, their tails sterilized with an alcohol swab and dried with tissue paper. A 21-G needle was advanced 1 cm into the tail vein at approximately 3 cm from the tail end and immediately withdrawn. Issuing

blood was carefully blotted every 5 s using the rough side of a filter paper (Qualitative filter paper No. 2; Advantec Toyo Kaisha, Ltd., Tokyo, Japan). When no further blood appeared on the filter paper, the bleeding was judged to have ceased and the measurement concluded. The number of bloodstains on the filter paper was counted, and bleeding time (s) was calculated by multiplying the total number of blood stains by 5.

Platelet transfusion experiment

Prasugrel (10 mg·kg⁻¹, p.o.) and ticagrelor (30 mg·kg⁻¹, p.o.), doses that produced similar levels of inhibition of platelet aggregation, were administered to rats 4 h before the bleeding time measurements. Fresh, washed platelets (1 × 10¹⁰ platelets·mL⁻¹) were prepared from other rats and suspended in Hank's balanced salt solution. Platelets (1 × 10¹⁰ platelets/rat) were transfused via the jugular vein to rats 1 h before the bleeding time measurements and the bleeding time was determined as described earlier. Red blood cell and platelet numbers in whole blood were measured just before platelet transfusion and after bleeding time measurements using an automated blood cell counter (KX-21N, Sysmex Corporation).

Data analysis

Platelet aggregation, thrombus weight and bleeding time were expressed as means ± SEM. Dunnett's multiple comparison test was carried out for platelet aggregation, thrombus weight and bleeding time at each time point using the vehicle group as control. In platelet transfusion experiments, a *t*-test was performed for the prasugrel and ticagrelor groups without platelet transfusion using the vehicle group as a control, and was also performed between the groups with and without platelet transfusion in prasugrel- and ticagrelor-treated rats. In all analyses, statistical significance was defined as *P* < 0.05. IC₅₀, ED₅₀ and ED₂₀₀ values were calculated from the regression line for dose–response relationship for inhibition of platelet aggregation, thrombus weight and bleeding time for each test article. SAS 8.2 and 9.2 for Windows (SAS Institute Inc., Cary, NC, USA) and EXSUS Ver. 7.1.6 and 7.7.1 (Arm Systex Co., Ltd., Osaka, Japan) or GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA, USA) were used to test significance and calculate ED₅₀ or IC₅₀ values.

Materials

Prasugrel hydrochloride and R-138727 were synthesized by Ube Industries, Ltd. (Yamaguchi, Japan). Ticagrelor was synthesized by Chemtech Labo, Inc. (Tokyo, Japan). AR-C124910XX was synthesized by Daiichi Sankyo RD Novare Co., Ltd. (Tokyo, Japan). Prasugrel and ticagrelor were suspended in 5% (w/v) solution of gum Arabic (Wako Pure Chemical Industries, Osaka, Japan). Prasugrel, ticagrelor and vehicle (5% gum Arabic solution) were orally administered to non-fasted rats in a volume of 1 mL·kg⁻¹. The source of other reagents was as follows: ADP sodium salt and collagen (LMS Co., Ltd., Tokyo, Japan).

Results

ADP-induced platelet aggregation in vitro

In the vehicle group of each test agent, mean platelet aggregations ranged from 52 to 59% for 5 µmol·L⁻¹ ADP

Table 1

IC₅₀ values (95% confidence intervals) for each P2Y₁₂ receptor antagonist for *in vitro* platelet aggregation in rat PRP, induced by ADP

Agents	IC ₅₀ (µmol·L ⁻¹) with 95% confidence interval	
	ADP 5 µmol·L ⁻¹	ADP 20 µmol·L ⁻¹
R-138727	18 (16–20)	24 (22–27)
Ticagrelor	0.82 (0.75–0.91)	1.5 (0.99–2.1)
AR-C124910XX	0.34 (0.14–0.50)	0.52 (0.39–0.67)

Test agents were incubated with rat PRP for 30 min *in vitro*, and platelet aggregation induced by ADP was assessed. R-138727 is the active metabolite of prasugrel and AR-C124910XX is an active metabolite of ticagrelor.

and 67–74% for 20 µmol·L⁻¹ ADP. R-138727 (prasugrel's active metabolite, 0.30–300 µmol·L⁻¹), ticagrelor (0.030–30 µmol·L⁻¹) and AR-C124910XX (ticagrelor's active metabolite, 0.030–30 µmol·L⁻¹) inhibited ADP- (5 and 20 µmol·L⁻¹) induced platelet aggregation in a concentration-related manner. The IC₅₀ values (with 95% confidence intervals) are summarized in Table 1. In the present study, the *in vitro* effect of prasugrel was not tested because prasugrel is a prodrug and thus has no *in vitro* effect on platelet aggregation (Sugidachi *et al.*, 2000).

Time course of ex vivo platelet aggregation induced by ADP

Ex vivo platelet aggregation was used to measure the effects of single oral doses of prasugrel and ticagrelor on platelet aggregation induced by 5 and 20 µmol·L⁻¹ ADP and were determined in blood samples taken at 1, 2, 4, 8, 12 and 24 h after administration. For platelet aggregation induced by 20 µmol·L⁻¹ ADP, single oral administration of prasugrel (0.3–3 mg·kg⁻¹) caused dose-related inhibitory effects (Figure 1A). With 1 and 3 mg·kg⁻¹ of prasugrel, significant inhibition was observed from 1 to 2 h after dosing. This inhibitory effect peaked at 4 h and lasted for 24 h after dosing, indicating a long duration of action by prasugrel. Single oral administration of ticagrelor (1–10 mg·kg⁻¹) also caused dose-related inhibitory effect on platelet aggregation (Figure 1B). Ticagrelor, at the highest dose (10 mg·kg⁻¹) significantly inhibited platelet aggregation at 1 h after dosing and the peak inhibition was observed at 4 h after dosing. The inhibitory effect on platelet aggregation lasted for 12 h after the dosing, but had returned to control values by 24 h, indicating a shorter duration of antiplatelet action than that of prasugrel. For platelet aggregation induced by 5 µmol·L⁻¹ ADP, similar time courses of inhibition of platelet aggregation were observed for both prasugrel- and ticagrelor-treated rats (data not shown), although the level of inhibition of platelet aggregation was slightly greater compared with that observed with 20 µmol·L⁻¹ ADP. The ED₅₀ values for prasugrel and ticagrelor at 4 h after the dosing were 1.5 mg·kg⁻¹ and 6.0 mg·kg⁻¹, respectively, for 5 µmol·L⁻¹ ADP and 1.9 mg·kg⁻¹ and 8.0 mg·kg⁻¹, respectively, for 20 µmol·L⁻¹ ADP.

ADP concentration-response for *ex vivo* platelet aggregation

The inhibitory effects of prasugrel and ticagrelor on platelet aggregation induced by higher concentrations of ADP (50 and 200 $\mu\text{mol}\cdot\text{L}^{-1}$) were also determined at the time of peak effect, 4 h after dosing. Prasugrel (1 or 3 $\text{mg}\cdot\text{kg}^{-1}$) significantly inhibited platelet aggregation induced by ADP at all concentrations tested in a dose-related manner, and the effect

was not reversed by increasing the concentration of ADP (Figure 2). Ticagrelor (3 or 10 $\text{mg}\cdot\text{kg}^{-1}$) also showed significant inhibition of platelet aggregation induced by ADP at all concentrations used, and, similarly, the effect of ticagrelor on ADP-induced aggregation appeared to be insurmountable, with no tested concentration of ADP completely overcoming the inhibition (Figure 2).

Collagen-induced *ex vivo* platelet aggregation

In addition to ADP-induced platelet aggregation, inhibitory effects of prasugrel and ticagrelor on collagen-induced platelet aggregation (5 $\mu\text{g}\cdot\text{mL}^{-1}$) were determined 4 h after dosing. Both prasugrel (0.3–3 $\text{mg}\cdot\text{kg}^{-1}$) and ticagrelor (1–10 $\text{mg}\cdot\text{kg}^{-1}$) inhibited collagen-induced platelet aggregation in a dose-related manner, with significant inhibitory effects being observed after 1 or 3 $\text{mg}\cdot\text{kg}^{-1}$ prasugrel and 3 or 10 $\text{mg}\cdot\text{kg}^{-1}$ ticagrelor (Figure 3).

AV shunt thrombosis

The anti-thrombotic effects of prasugrel and ticagrelor *in vivo* were assessed in the rat AV shunt thrombosis model. Prasugrel (0.3–3 $\text{mg}\cdot\text{kg}^{-1}$) and ticagrelor (1–10 $\text{mg}\cdot\text{kg}^{-1}$) were orally administered, and blood was allowed to circulate through the shunt 4 h after dosing. Thrombus weight in the vehicle-treated group was 45.9 ± 1.3 mg. Prasugrel significantly reduced thrombus weight at doses of 1 and 3 $\text{mg}\cdot\text{kg}^{-1}$ in a dose-related manner, compared with that in the vehicle-treated group with an ED_{50} value of 1.8 $\text{mg}\cdot\text{kg}^{-1}$. Ticagrelor also significantly reduced the thrombus weight at doses of 3 and 10 $\text{mg}\cdot\text{kg}^{-1}$ with an ED_{50} value of 7.7 $\text{mg}\cdot\text{kg}^{-1}$ (Figure 4A).

Bleeding time

Effects of prasugrel (0.3–3 $\text{mg}\cdot\text{kg}^{-1}$) and ticagrelor (0.3–10 $\text{mg}\cdot\text{kg}^{-1}$) on the bleeding time in rats were determined 4 h after administration of vehicle, prasugrel or ticagrelor (Figure 4B). Prasugrel and ticagrelor each significantly prolonged bleeding time at 1 $\text{mg}\cdot\text{kg}^{-1}$ or higher doses, compared with the vehicle group (84 ± 4 s). Doses causing a twofold increase of the bleeding time in the vehicle group were calculated by linear regression analysis; the dose was estimated

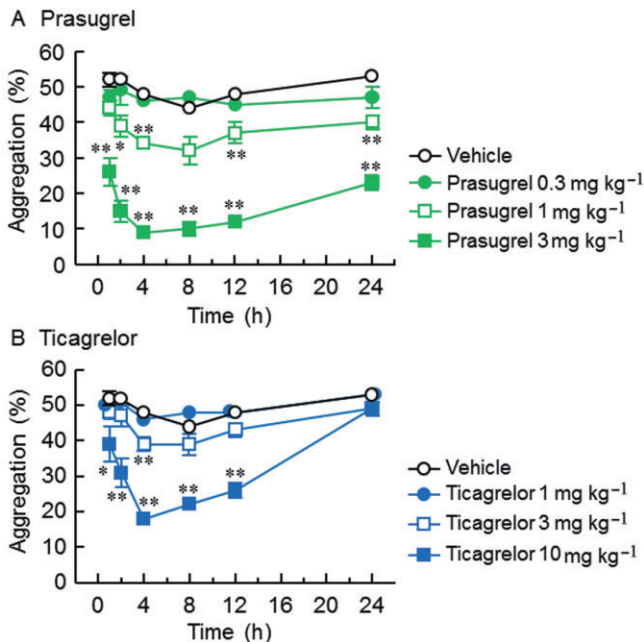


Figure 1

Ex vivo effects of single doses of prasugrel (A) or ticagrelor (B) on platelet aggregation induced by ADP in rats. Agents were orally administered to rats and blood was collected 1, 2, 4, 8, 12 and 24 h after dosing. *Ex vivo* platelet aggregation in PRP was induced by 20 $\mu\text{mol}\cdot\text{L}^{-1}$ ADP. Results are presented as the mean \pm SEM ($n = 5$). * $P < 0.05$, ** $P < 0.01$, significantly different from vehicle (Dunnett's test).

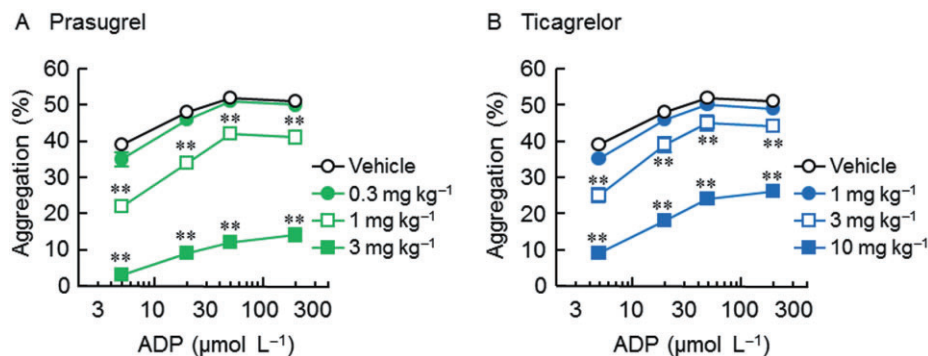


Figure 2

Concentration-response curve for ADP-induced platelet aggregation in prasugrel- (A) and ticagrelor-treated rats (B). Prasugrel and ticagrelor were orally administered to rats 4 h before blood collection. *Ex vivo* platelet aggregation in PRP was induced by 5, 20, 50 and 200 $\mu\text{mol}\cdot\text{L}^{-1}$ ADP. Results are presented as the mean \pm SEM ($n = 5$). ** $P < 0.01$, significantly different from vehicle group (Dunnett's test).

without logarithmic transformation of the bleeding time. These values (ED_{200}) were $3.0 \text{ mg}\cdot\text{kg}^{-1}$ for prasugrel and $13 \text{ mg}\cdot\text{kg}^{-1}$ for ticagrelor.

Platelet transfusion study

Both prasugrel ($10 \text{ mg}\cdot\text{kg}^{-1}$) and ticagrelor ($30 \text{ mg}\cdot\text{kg}^{-1}$) significantly prolonged the bleeding time compared with vehicle-treated control groups ($P < 0.001$ and $P < 0.01$, respectively) (Figure 5). Platelet transfusion at 3 h after prasugrel or

ticagrelor dosing resulted in similar significant increases (1.61- and 1.56-fold, respectively) in blood platelet numbers in prasugrel- and ticagrelor-treated rats (data not shown). In contrast, red blood cell numbers were not changed by platelet transfusion in either group (data not shown). In the prasugrel-treated group, platelet transfusion resulted in significant shortening of bleeding time ($P < 0.05$, Figure 5). In the ticagrelor-treated group, by contrast, platelet transfusion did not change bleeding time ($P > 0.05$, Figure 5). Thus, while the prolongation of bleeding time induced by high-dose of prasugrel could be significantly reversed by platelet transfusion, this was not the case for ticagrelor.

Discussion and conclusions

Thienopyridines including prasugrel are antiplatelet prodrugs and their action is mediated by their active metabolites generated *in vivo* (Savi *et al.*, 2000; Sugidachi *et al.*, 2000). In contrast, ticagrelor itself has antiplatelet activity (Springthorpe *et al.*, 2007) and Sillén *et al.* (2010) have reported recently that ticagrelor also has an *in vivo* active metabolite AR-C124910XX, with potency similar to that of ticagrelor (van Giezen and Humphries, 2005; Teng and Butler, 2010). To our knowledge, however, there is no detailed report describing the antiplatelet activity of ticagrelor's active metabolite. The present study is the first report showing antiplatelet activity of AR-C124910XX, which was 2.4- to 2.9 times more potent than its parent, ticagrelor, in rat PRP. In addition, our preliminary experiment showed similarly potent activity of AR-C124910XX in human PRP (data not shown). The pharmacokinetic profile of AR-C124910XX in rats is not known, but, in humans, the $AUC_{0-\infty}$ of AR-C124910XX is about half to one-fifth of ticagrelor's $AUC_{0-\infty}$ (Teng and Butler, 2010; Husted *et al.*, 2012). Taken together, therefore, these results suggest that clinically, both

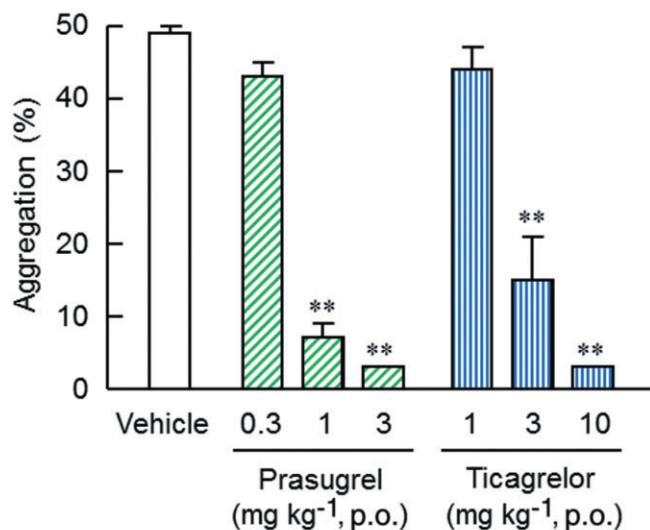


Figure 3

Ex vivo effects of prasugrel or ticagrelor on collagen-induced platelet aggregation in rats. Prasugrel and ticagrelor were orally administered to rats 4 h before blood collection. *Ex vivo* platelet aggregation in PRP was induced by $5 \mu\text{g}\cdot\text{mL}^{-1}$ collagen. Results are presented as the mean + SEM ($n = 5$). ** $P < 0.01$, significantly different from vehicle group (Dunnett's test).

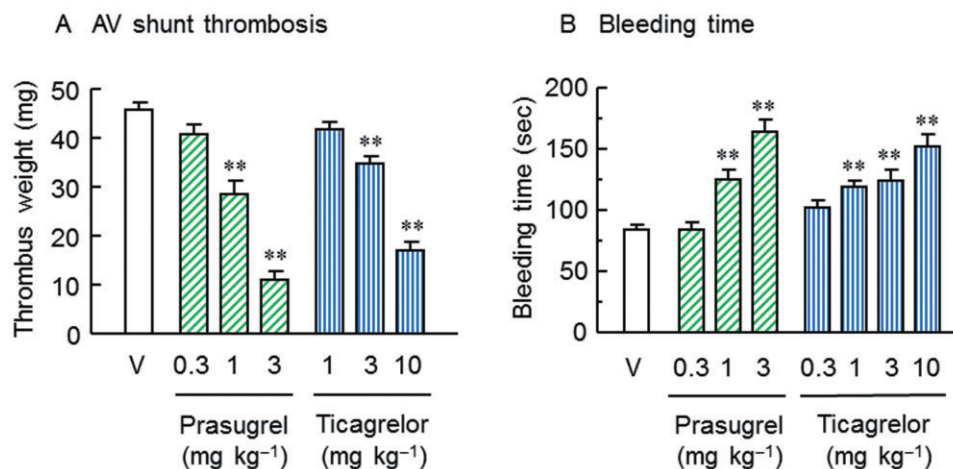


Figure 4

Effects of prasugrel or ticagrelor on AV shunt thrombosis (A) and bleeding time (B) in rats. Prasugrel and ticagrelor were orally administered to rats 4 h before circulation of blood through the AV shunt or bleeding time measurements. Results are presented as the mean + SEM ($n = 10$). V = vehicle control. ** $P < 0.01$, significantly different from vehicle group (Dunnett's test).

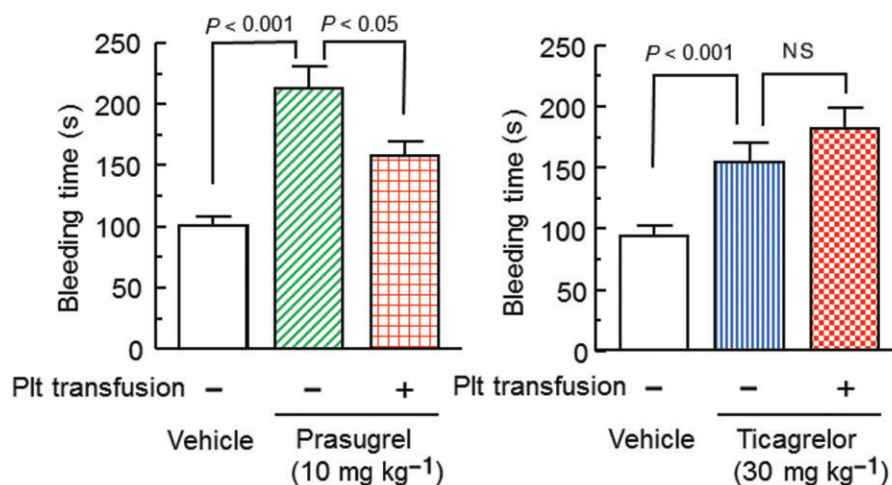


Figure 5

Effects of platelet transfusion on prolongation of bleeding time by prasugrel or ticagrelor in rats. Prasugrel and ticagrelor were orally administered 4 h before bleeding time measurements. Platelets (Plt) were infused i.v. 1 h before bleeding time measurements. Bleeding times are shown as the means + SEM ($n = 13$ – 14). Plt, platelets; NS, not significant (t -test).

ticagrelor and AR-C124910XX would have approximately equivalent effects on *in vivo* platelet inhibition following ticagrelor dosing.

In the present study, prasugrel's active metabolite (R-138727) inhibited *in vitro* platelet aggregation, and its *in vitro* antiplatelet activity was less potent than ticagrelor and AR-C124910XX. Nevertheless, as reported in clinical studies (Jernberg *et al.*, 2006; Gurbel *et al.*, 2010), on a $\text{mg}\cdot\text{kg}^{-1}$ basis, orally administered prasugrel was more potent than ticagrelor on *ex vivo* platelet aggregation induced by ADP. In addition, we found prasugrel's inhibition of *ex vivo* platelet aggregation was sustained for up to 24 h after the administration, while ticagrelor demonstrated a shorter duration of action and the antiplatelet effect was lost 24 h after dosing. Possible explanations for these differences between *in vitro* and *ex vivo* effects may be due to differences in pharmacokinetic profiles and their different irreversible (Sugidachi *et al.*, 2000; 2001) and reversible antiplatelet actions (Wijeyeratne *et al.*, 2012). Irreversible inhibition of P2Y₁₂ receptors by prasugrel is also an explanation of the longer duration of antiplatelet action. In addition, a recent report showed that ticagrelor may have effects on adenosine transporters in human platelets, which may contribute to its antiplatelet action (Iyú *et al.*, 2011). Thus, inhibition of adenosine transporters may contribute to the more potent *in vitro* activity of ticagrelor. However, another report suggested that any effect of ticagrelor on adenosine uptake was not enough to amplify the antiplatelet effects of any adenosine generated in the presence of P2Y₁₂ receptor antagonists (van Giezen *et al.*, 2012). Furthermore, the ADP concentration/response aggregation profiles of prasugrel and ticagrelor were compared at peak inhibition of platelet aggregation, and found to be similar with both agents, as previously reported (Sugidachi *et al.*, 2000; van Giezen *et al.*, 2009), acting as non-competitive antagonists.

Antithrombotic therapy is a cornerstone of treatment in patients with cardiovascular disease with bleeding being the

most worrisome complication. Indeed, while prasugrel and ticagrelor provide greater inhibition of platelet aggregation and clinical efficacy than clopidogrel, both agents were associated with more bleeding compared with clopidogrel (Wiviott *et al.*, 2007; Becker *et al.*, 2011). In the present study, the relationship among antiplatelet, antithrombotic and bleeding activities of both prasugrel and ticagrelor were compared in rats. Both agents inhibited platelet aggregation, thrombus formation and haemostasis in a dose-related manner with a similar potency ratio of about four times among all the parameters tested at the time of peak inhibition of platelet aggregation (4h). These data suggest that at equivalent levels of platelet inhibition, the two agents would show similar antithrombotic activity with similar bleeding risk despite their distinct modes of actions at the P2Y₁₂ receptor level, that is reversible (ticagrelor) or irreversible (prasugrel) antagonism.

There are several reversal strategies available for patients on antiplatelet therapy who present with an acute haemorrhage or require urgent surgery, including platelet transfusion (Lemmer, 2000; McMillian and Rogers, 2009), although some potentially deleterious effects have been observed (Bassand, 2009). Indeed, Vilahur *et al.* (2007) reported that *in vitro* platelet concentrates could restore haemostatic potential in the face of clopidogrel-induced platelet dysfunction. The present *in vivo* result showed that platelet transfusion significantly shortened prasugrel-induced prolongation of bleeding time in rats. In contrast, in ticagrelor-treated rats, platelet transfusion failed to reverse prolongation of bleeding time. This unexpected finding may depend on the different reversibility profile and/or different pharmacokinetic profile of the agent: It would appear that antiplatelet action of ticagrelor correlates with blood levels of ticagrelor and its active metabolite (Teng and Butler, 2010; Husted *et al.*, 2012). Thus, newly transfused platelets would be readily inhibited by the presence of free ticagrelor and/or its active metabolite in plasma. In contrast, prasugrel is an irreversible antiplatelet

agent with only transient exposure of platelets to its active metabolite needed for sustained platelet inhibition. At 4 h after the dosing, when maximum inhibition of platelet aggregation was observed in rats, the blood concentration of prasugrel's active metabolite is significantly lower than its peak (C_{max}) level (Sugidachi *et al.*, 2007; Hagihara *et al.*, 2009), thereby allowing transfused platelets to remain functional and provide haemostatic potential. Furthermore, this pharmacokinetic / pharmacodynamic relationship in prasugrel-treated rats is similar to that observed in humans (Jakubowski *et al.*, 2007).

In conclusion, both prasugrel and ticagrelor inhibited platelet aggregation and thrombus formation while prolonging bleeding with a similar potency ratio of approximately four times among these activities. In addition, prasugrel showed longer duration of antiplatelet action compared with ticagrelor. The present study also suggested that ticagrelor and its active metabolite might play equal roles in providing *in vivo* antiplatelet activity. Although the platelet inhibitory effect of prasugrel was reversed by platelet transfusion, that of ticagrelor was not. The disparity in findings between prasugrel- and ticagrelor-treated rats may reflect the different reversibility and/or pharmacokinetic profiles of the two agents.

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Conflict of interest

J. A. Jakubowski is an employee of Eli Lilly and Company. Other authors are employees of Daiichi Sankyo Co., Ltd.

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